Biol. Stud. 2025, 19(3), 67–76 • doi: https://doi.org/10.30970/sbi.1903.843
www.http://publications.lnu.edu.ua/journals/index.php/biology

UDC: 576.316:636.293.2(477)

CYTOGENETIC STUDY OF THE UKRAINIAN RIVER BUFFALO (*BUBALUS BUBALIS BUBALIS*)

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Dzitsiuk, V., Ryk, T., & Lytvynenko, T. (2025). Cytogenetic study of the Ukrainian river buffalo (*Bubalus bubalis bubalis*). *Studia Biologica*, 19(3), 67–76. doi:10.30970/sbi.1903.843

Introduction. The article presents the results of the exploration of the karyotype of the Ukrainian population of river buffaloes (*Bubalus bubalis bubalis*), which are bred in Ukraine in the household of Sviato-Pokrovska Holosiivska Pustyn monastery.

Materials and Methods. Chromosome preparations were obtained from cultured peripheral blood lymphocytes. Whole venous blood (5 mL) was cultured for 48 h at 37 °C in RPMI 1640 medium (Sigma, USA) supplemented with 0.1 mL/mL phytohemagglutinin (PHA, Sigma, USA) and 15% fetal calf serum. Colchicine (Serva, Germany, 10 µg/mL) was added 2 h before harvest to arrest cell division. Chromosomal preparations were stained with 2% Giemsa solution (Merck) and analyzed at 1000x magnification using an Axiostar plus microscope (Carl Zeiss, Germany). Routine, G-, and Ag-banding were performed to determine the spontaneous rate of chromosome aberrations and the level of chromosomal variability. Metaphase spreads were photographed with an Olympus D-460 ZOOM digital camera. Statistical analysis was performed using Microsoft Excel 2010.

Results. The diploid chromosomal set of the studied animals consists of 50 chromosomes (2n = 48, XX; 2n = 48, XY). Aneuploid and polyploid cells were discovered at rates 7.70±1.59 % and 0.35±0.053 % respectively, as well as cells with structural aberrations of autosomes. The overall rate of cells with dysfunctions amounted to 12.55±2.00 %. The rate of chromosomal aberrations in males and females differed insignificantly and amounted to 12.60 % and 11.30 % respectively. We detected

individuals with cells with monosomy X0. Ag-banding revealed nucleolus organizer regions (NOR) in six chromosomes of the karyotype of the studied buffaloes – 3, 4, 6, 21, 23, 24. Individual variation of animals by the NOR number was deduced from 1 to 12 per cell, with an average of 2,82.

Conclusions. Cytogenetic research can be used for the preservation of diversity and improvement of breeding programs for the Ukrainian river buffalo population.

Keywords: river buffalo, karyotype, chromosomes, aberrations

INTRODUCTION

There are two main species of buffalo in the world: the Asian buffalo (*Bubalus bubalis*), often referred to as the water or river buffalo, and the African buffalo (*Syncerus caffer*). The Asian buffalo *Bubalus bubalis* is further classified into two main subspecies: the river buffalo (karyotype 2n = 50, FN = 60) and the swamp buffalo (2n = 48, FN = 58) (Iannuzzi, 2003). These subspecies exhibit a notable chromosomal difference: the river buffalo has 50 chromosomes, while the swamp buffalo has 48. This difference arises from a centric fusion, where chromosomes 4 and 9 of the river buffalo have fused to form what is now chromosome 1 of the swamp buffalo. Despite this karyotypic divergence, all chromosome arms between these two Asian subspecies are largely conserved, allowing them to interbreed and produce viable, although often less fertile, F1 offspring with 49 chromosomes (Iannuzzi, 2003; Cailipan *et al*., 2023).

In addition to basic karyotyping, recent advances in genome sequencing of water buffaloes have provided a deeper understanding of their genetic structure and evolutionary relationships. Recent progress in genome sequencing, including the generation of high-quality reference genomes for Bubalus bubalis, has greatly expanded our understanding of genetic traits of economic importance, as well as the evolutionary relationships among buffalo populations (Rehman, 2021). In addition, valuable information regarding genetic diversity, population structure, and genes associated with economically important traits such as milk production and disease resistance has been revealed. Similarly, efforts to sequence the genome of the swamp buffalo (Li, 2018) have shed light on the unique evolutionary pathways of these diverse groups. These genomic studies complement traditional cytogenetic ones by offering a more detailed view of chromosomal rearrangements, gene content, and genetic relationships, further confirming the distinctness and evolutionary divergence of river and swamp buffaloes, with the latter showing closer relationships with other species, such as the tamarao buffalo (Bubalus mindorensis) (Cailipan *et al*., 2023).

Buffaloes in Ukraine are a domesticated form of the Asian buffalo *Bubalus bubalis* – river buffalo. This is the northernmost branch of Asian buffalo settlement in the world (Castelló, 2016).

The earliest records of buffaloes in Ukraine date to 1819, noting their use in transporting salt at salt mines. In pre-Soviet Zakarpattia, buffaloes were commonly raised as a substitute for cows, serving multiple purposes: draft animals, a source of meat, milk, leather, and bone, and importantly, for their manure. Buffalo manure was utilized in housing construction, as a heating fuel, and to improve soil fertility. With the advent of collectivization, buffaloes, along with cattle, were incorporated into collective farms, under which they did not survive (Guzeev, 2014).

Since 2007, the transportation of buffaloes from Zakarpattia to Kyiv Region has begun. The “Holosievo” farm in the Brovar District of Kyiv Region has become a model household for buffalo breeding (60 heads of different sexes and ages).

Research on the genetic diversity of buffaloes in Ukraine is especially relevant because these animals have been insufficiently studied. Only individual reports about research on the genome of river buffalo exist (Guzeyev *et al.*, 2016; Dzitsiuk *et al.*, 2020). Therefore, the present study aims to examine the karyotype of river buffaloes (river buffalo) bred in Ukraine.

MATERIALS AND METHODS

The material for the study was the peripheral blood of 55 adult river buffaloes (26 males and 29 females), which are held in the household of Sviato-Pokrovska Holosiivska Pustyn-Holosievo monastery.

To prepare chromosome preparations, whole venous blood (5 mL) was cultivated for 48 h at +37 °C in RPMI 1640 medium (Sigma, USA) with the addition of 0.1 mL/mL PHA (phytohemagglutinin, Sigma, USA), 15% embryonic calf serum. Colchicine (Serva, Germany, 10 μg/mL) was added two hours prior to the completion of the cultivation period to stop cell division. The precipitate of cells was obtained by centrifugation for 10 min at 1000 g and the subsequent treatment with hypotonic KCl solution (0.075 M) for 20 min. The fixation of cells was conducted in three changes of the methanol-acetic acid mixture in a 3:1 ratio. The cell suspension of the required density, obtained in the last portion of the fixator, was dropped on cooled and wet specimen slides and analyzed using a Zeiss Axiostar plus microscope (Germany) with a magnification of 10×100, using a 100× oil immersion objective with n. a. of 1.25. For routine chromosome staining, a 2% solution of Giemsa stain was used (ready-to-use product, Merck, KGaA, Germany). Chromosomes were identified by the differential staining method (G-banding) with the use of trypsin (Seabright, 1971).

Nucleolus organizer regions (NORs) on the metaphase chromosomes were detected by staining with a 50% solution of silver nitrate ($AgNO_3$) according to Ploton’s recommendations (Ploton *et al.*, 1986). Then, a 0.2% formic acid (pH 2.6–2.7) and a 50% solution of $AgNO_3$ in a ratio of 1:1 were applied to the object glass. The preparation was placed in a Petri dish on a moistened filter paper and kept in a thermostat for 5–8 min at 62 °C. NORs were detected on telomeres of corresponding chromosomes as dark dots.

The results were presented as the mean ± standard deviation, n – number of experiments. The digital material obtained was processed using standard methods of variational statistics from the Office Excel 2003 package.

RESULTS AND DISCUSSION

The diploid karyotype of river buffalo consists of 50 chromosomes (2n = 50, FN = 60) and has 5 pairs of metacentric and submetacentric autosomes and 19 pairs of acrocentric chromosomes. It is known that Asian buffalo (*Bubalus bubalis*) is cytogenetically dimorphic: its subspecies have different numbers of chromosomes in their karyotype – the river buffalo has 50 chromosomes, while the swamp buffalo – 48 chromosomes. Iannuzzi (Iannuzzi *et al.*, 2005) explains it by the fact that, as a result of the evolutionary process, the first chromosome of river buffalo is formed by the translocation of the fourth

the ninth chromosomes. Despite this, the entire species gene set of the buffalo karyotype is maintained. Subspecies of river and swamp buffaloes interbreed and produce offspring with an odd number of chromosomes – 49.

The sex X chromosome is the largest acrocentric chromosome, which can be identified by routine staining. This chromosome has the morphological distinction – a membrane in the long arm, which makes it easy to differentiate it from the others. The Y chromosome is the smallest acrocentric chromosome (Degrandi *et al*., 2014).

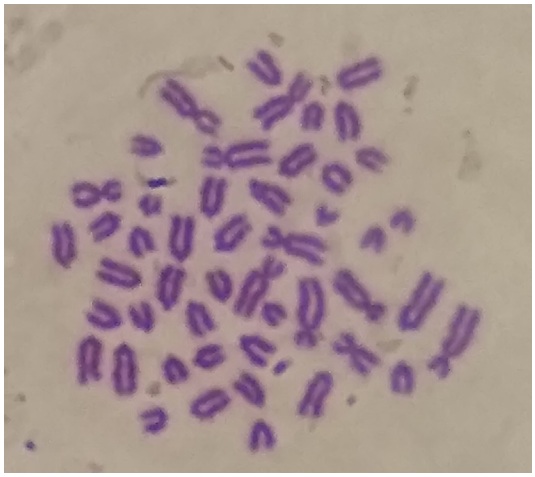

Fig. 1. Typical metaphase plates of the river buffalo (2n = 50). Magnification: vol. ×100; circ. ×10

In the studied karyotypes of buffaloes, numerical and structural aberrations of aneuploidy, polyploidy, fractures, and fragments of chromosomes (see **Table**) were detected. Numerical aberrations of karyotype constituted the largest category of aberrations – aneuploidy and polyploidy (in the amount of 8.05 %). Structural aberrations of chromosomes, such as fractures, fragments, and others, constitute 38 % of the total amount of karyotype abnormalities.

Frequency of chromosomal aberrations in lymphocytes of river buffaloes

Animals studied		55
Studied metaphases		1750
Aberrant cells, %		12.05±1.80
Including:		
Frequency of genomic aberrations, %	Aneuploid cells	7.70±1.59
	Polyploid cells	0.35±0.05
Frequency of structural chromosomal aberrations, %	Breaks	2.40±0.09
	Fragments	2.30±0.08

The proportion of aneuploid cells in the general spectrum of aberrations in the studied buffaloes was greater than half and was represented by hypoploid cells (2n = 48–49) with a minor number of hyperploid cells (2n = 51–52). Polyploid cells were encountered infrequently (0.35±0.05) and predominantly had a triploid set of chromosomes.

Structural aberrations in the karyoptype were represented by breaks and fragments of chromosomes. In aberration, separate chromosomes from the second, third, fifth, and seventh pairs were engaged the most frequently. No breaks, deletions, or duplications in small-sized chromosomes were detected.

The obtained results are somewhat different from cytogenetic study data, conducted at the National Research Center (Department of Cell Biology, Egypt) (Ahmed, 2005). In our research, the frequency of cells with chromosomal breaks was 2.40 %, which is almost half as much as reported by S. Ahmed (4.4 %).

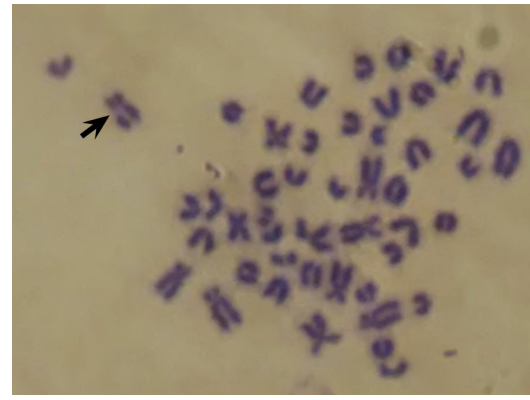

Fig. 2. Metaphase plate of river buffalo with a chromosome that contains a break (indicated by an arrow). Magnification: vol. ×100; circ. ×10

In contrast to cattle, chromosome translocations were not found in the studied buffaloes. However, in some animals, the association of two autosomes with the formation of an S-shaped form was observed.

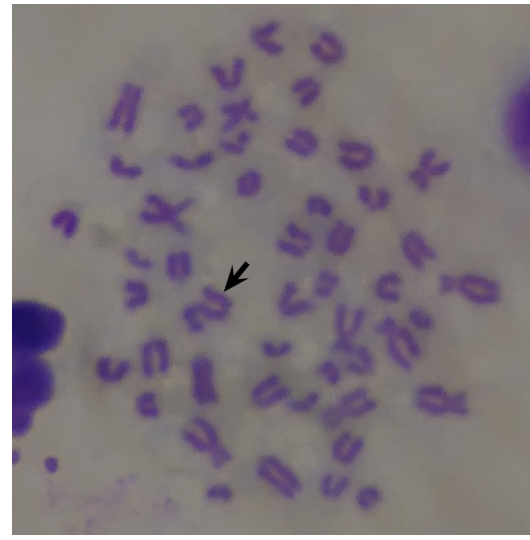

Fig. 3. Metaphase plate with an S-shaped chromosome. Magnification: vol. ×100; circ. ×10

The frequency of cells with aberrations in females and males did not differ significantly and amounted to 12.60 % and 11.30 % respectively. However, in females, cells with chromosomal fragments predominated, whereas males had more cells with broken chromosomes.

The frequency of aneuploid and polyploid cells in both sex groups showed no significant difference (**Fig. 4**).

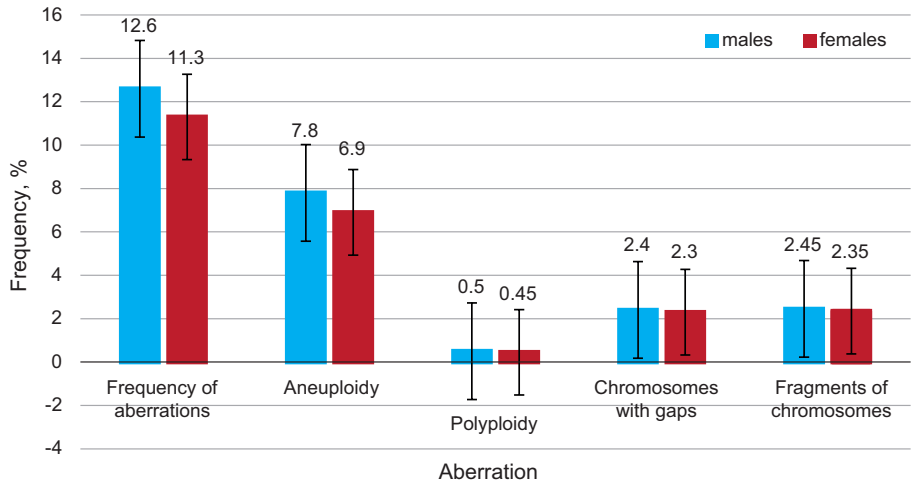

Fig. 4. Frequency of chromosomal aberrations in different sex groups of river buffaloes

The number of chromosomal aberrations was found to be statistically similar in females and males, accounting for 12.69 % in adult females and 11.30 % in breeding male buffaloes, respectively.

The reports about aberrations in the karyotype of river buffaloes, related to sex chromosomes, are often found in the literature: X-trisomy (Iannuzzi *et al*., 2004); X-monosomy (Iannuzzi *et al*., 2000), XXY chromosome complement due to X-X-translocation (Patel *et al*., 2015).

In another female with a normal body structure and reproductive capacity, the coexistence of two genetically different cell populations was detected. Two types of cells were identified under the microscope: 48, XX and 48, XO. Similar cases of aneuploidy, provoked by the absence of one of the X chromosomes, are also described in the literature (Iannuzzi *et al*., 2021).

Using the Ag-banding technique, active nucleolar organizers (Nucleolus Organizer Regions, NORs) were detected on six chromosomal pairs in river buffaloes: 3, 4, 6, 21, 23, and 24 (**Fig. 5**). These data are consistent with standard nomenclature, which indicates that NORs in river buffaloes are located on the telomeres of six chromosomal pairs: 3p, 4p, 6, 21, 23, and 24 (Iannuzzi *et al*., 2021).

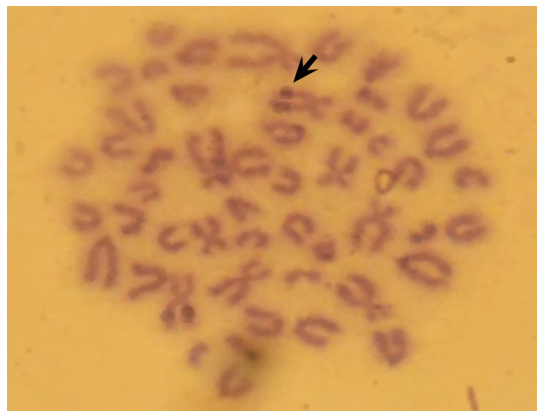

Fig. 5. Karyotype of a river buffalo with active NORs at chromosome telomeres

The research discovered that the number of active NORs in cells varies from 1 to 12. The largest proportion of cells had two NORs (45 %), with half as many having three nucleolus organizers. Cells with one and four NOR constituted 12 % each. The cells with nucleolus organizers from 6 to 12 account for about 1 % (**Fig. 6**).

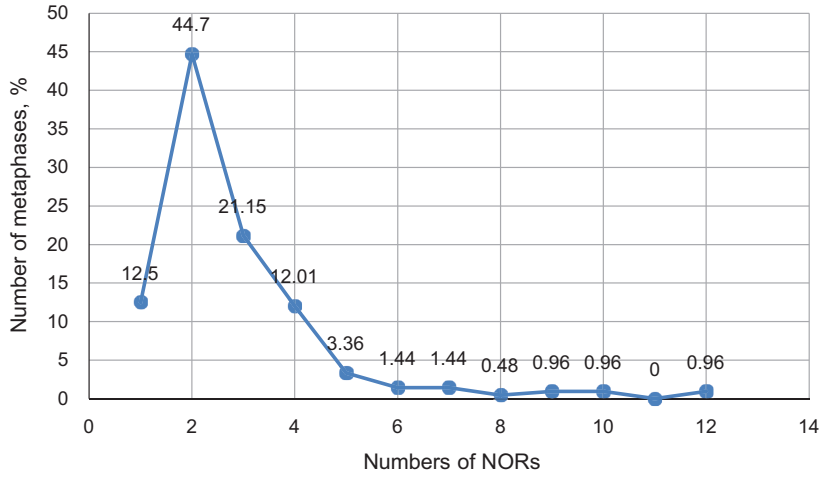

Fig. 6. Number of metaphases with different numbers of NOR in the chromosomes of river buffalo

CONCLUSIONS

The research on buffaloes of the Ukrainian population has established that their karyotype is within normal limits: 2n = 48+XX in females and 2n = 48+XY in males. The spontaneous level of chromosomal variation was observed at 12.55±2.00 % due to aneuploid and polyploid cells with the frequency of 7.70±1.59 % and 0.35±0.053 % respectively, as well as cells with structural aberrations of autosomes. The frequency of chromosomal aberrations in males and females did not differ significantly, amounting to 12.60 % and 11.30 % respectively. Through the use of the G-banding technique, individuals with cells with monosomy X0 and monosomy of the second chromosomal pair, as well as cells with chromosomal breaks in the 3rd pair, were detected. The Ag-banding technique revealed nucleolus organizer regions (NORs) on six chromosome pairs – 3, 4, 6, 21, 23, and 24 – in the karyotype of the studied buffaloes.

The low number of buffaloes in Ukraine, contrasting with the high quality of products supported by them, emphasizes the critical importance of preserving this unique population. The results of genetic studies, particularly cytogenetic analyses, demonstrate the presence of genetic diversity – a key factor in developing effective conservation programs and restoring populations.

COMPLIANCE WITH ETHICAL STANDARDS

The authors received no financial support for the research, authorship, and publication of this article.

Conflict of interest: the authors declare that the study was conducted in the absence of any commercial or financial relationship that could be interpreted as a potential conflict of interest.

Human Rights: this article does not contain any research involving humans conducted by any of the authors

AUTHOR CONTRIBUTIONS

Conceptualization, [V.D]; methodology, [T.R.; T.L]; formal analysis, [V.D.].; resources, [V.D.; T.R.; T.L.]; data curation, [T.R.]; writing – original draft preparation, [V.D.; T.L.]; visualization, [T.R.]; project administration, [T.L.]; obtaining funding [-].

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ЦИТОГЕНЕТИЧНЕ ДОСЛІДЖЕННЯ БУЙВОЛА РІЧКОВОГО УКРАЇНСЬКОЇ ПОПУЛЯЦІЇ (*BUBALUS BUBALIS BUBALIS*)

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Вступ. У статті наведено результати дослідження каріотипу української популяції річкових буйволів (*Bubalus bubalis bubalis*), яких розводять в Україні у господарстві монастиря “Свято-Покровська Голосіївська Пустинь”.

Матеріали та методи. Хромосомні препарати отримували з культивованих лімфоцитів периферичної крові. Цільну венозну кров (5 мл) культивували протягом 48 год за 37 °C у середовищі RPMI 1640 (Sigma, США) з додаванням 0,1 мл/мл фітогемаглютиніну (PHA, Sigma, США) та 15% фетальної телячої сироватки. За 2 год до закінчення періоду культивування додавали колхіцин (Serva, Німеччина, 10 мкг/мл), щоб зупинити поділ клітин. Хромосомні препарати фарбували 2% розчином Гімзи (Giemsa, Merk) і аналізували під мікроскопом Axiostar plus (Carl Zeiss, Німеччина) на 1000-кратному збільшенні. Для визначення спонтанної частоти хромосомних аберацій і рівня хромосомної варіабельності лімфоцитів застосовували рутинне фарбування, G- та Ag-бендинг. Мікрофотографії метафазних пластинок отримували за допомогою цифрової камери Olympus D-460 ZOOM. Статистичну обробку даних проводили методами варіаційної статистики з використанням програмного пакета Microsoft Excel 2010.

Результати досліджень. Диплоїдний хромосомний набір досліджених тварин складається з 50 хромосом (2n = 48, XX; 2n = 48, XY). Виявлено анеуплоїдні та поліплоїдні клітини з частотою 7,70±1,59 % і 0,35±0,053 % відповідно, а також клітини зі структурними аберраціями аутосом. Загальний показник аберантних клітин становив 12,55±2,00 %. Частоти аберацій у самиць (12,60 %) і самців (11,30 %) статистично значуще не відрізнялася. Ідентифіковано особин із моносомією X0. Ag-banding дав змогу ідентифікувати ділянки ядерцевих організаторів (ЯОР) на шести хромосомах: 3, 4, 6, 21, 23 та 24. Спостерігали індивідуальну варіабельність ЯОР (від 1 до 12 на клітину), зі середнім значенням 2,82.

Висновки. Результати цитогенетичного дослідження можуть бути використані для збереження генетичного різноманіття й оптимізації програми розведення української популяції річкових буйволів.

Ключові слова: буйвол річковий, каріотип, хромосоми, аберації

Received / Одержано 20 January 2024 | Revision / Доопрацьовано 26 July, 2025 | Accepted / Прийнято 17 September, 2025 | Published / Опубліковано 24 September, 2025